INFECTION AND IMMUNITY, Apr. 2005, p. 2452–2460 Vol. 73, No. 4
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.4.2452–2460.2005

Human Leukocyte Antigen-DQ8 Transgenic Mice: a Model To Examine the Toxicity of Aerosolized Staphylococcal Enterotoxin B

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Received 29 July 2004/Returned for modification 11 October 2004/Accepted 30 November 2004

Staphylococcal enterotoxins (SEs) belong to a large group of bacterial exotoxins that cause severe immunopathologies, especially when delivered as an aerosol. SEs elicit the release of lethal amounts of cytokines by binding to major histocompatibility complex (MHC) class II and cross-linking susceptible T-cell receptors. Efforts to develop effective therapeutic strategies to protect against SEs delivered as an aerosol have been hampered by the lack of small animal models that consistently emulate human responses to these toxins. Here, we report that human leukocyte antigen-DQ8 (HLA-DQ8) transgenic (Tg) mice, but not littermate controls, succumbed to lethal shock induced by SEB aerosols without potentiation. Substantial amounts of perivascular edema and inflammatory infiltrates were noted in the lungs of Tg mice, similar to the pathology observed in nonhuman primates exposed by aerosol to SEB. Furthermore, the observed pathologies and lethal shock correlated with an upsurge in proinflammatory cytokine mRNA gene expression in the lungs and spleens, as well as with marked increases in the levels of proinflammatory circulating cytokines in the Tg mice. Unlike the case for littermate controls, telemetric evaluation showed significant hypothermia in Tg mice exposed to lethal doses of SEB. Taken together, these results show that this murine model will allow for the examination of therapeutics and vaccines developed specifically against SEB aerosol exposure and possibly other bacterial superantigens in the context of human MHC class II receptors.

Staphylococcus aureus and group A streptococci are responsible for a wide range of mild to life-threatening infections, including scarlet fever, pharyngitis, dermatitis, infectious arthritis, and toxic shock syndrome (2, 13, 18, 25, 31). These pathogenic bacteria use several virulence mechanisms to enhance their toxicity after infection, including the M protein, diffusible enzymes (e.g., DNase), and streptolysin. Furthermore, many strains of *S. aureus* produce bacterial superantigens (BSAgs), which exert a series of critical, negative immunological effects on the host. Specifically, BSAgs bind to major histocompatibility complex (MHC) class II molecules and form a ternary complex with receptive variable β chains of T-cell antigen receptors. After binding, BSAg-stimulated T cells are eliminated by a Fas/Fas-ligand-mediated apoptosis or, alternatively, enter a state of specific nonresponsiveness (anergy), which may last for several months. Furthermore, BSAgs may exacerbate subclinical viral infections by removing activated T cells from their normal role in clearing invasive organisms.

Mice are naturally insensitive to BSAg-induced lethal shock (23, 24, 32). In order to overcome the natural insensitivity of mice to staphylococcal enterotoxins (SEs), sublethal amounts of lipopolysaccharide (LPS) have been used to potentiate the lethal effects of BSAgs (7, 32). Although the exact mechanism of LPS induction is not known, it has been shown that lethality is dependent on the expression of mouse MHC class II molecules and synergistic cytokine responses to both LPS and BSAg (23, 24). Lethal shock was also observed when combinations of D-galactosamine, an agent known to disrupt liver metabolism (23, 24), and BSAgs were given to mice. Mouse resistance to bacterial BSAg toxicity may be due to a significantly lower affinity of the toxin for murine MHC class II molecules, which lack a critical lysine residue in the α chain (16). Nevertheless, mice have been used in BSAg toxicity assays, such as those measuring cytokine responses after potentiating doses of LPS, which enhances cytokine release. In fact, considerable evidence indicates that inflammatory cytokines, including gamma interferon (IFN-γ), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α), are chief participants in the systemic effects of the BSAgs (2, 8, 38). Compounds such as actinomycin D, LPS, and D-galactosamine are known to exacerbate cytokine actions or increase the biological half-lives of cytokines. Such small animal models may be satisfactory for testing vaccines, as it has been clearly demonstrated that lethality is prevented by SE-neutralizing antibodies (5, 8, 20). Since both BSAgs and the molecules used for potentiation in these models alter cytokine responses, it is difficult to tease apart the differential effects of the molecules and to interpret data obtained from these experiments. As both components of these models contribute to the pathogenesis of the toxic shock, this creates a significant limitation for assessing therapeutic compounds aimed specifically at treating BSAg-induced disease.

When delivered as an aerosol, small amounts of BSAgs can cause severe lung pathologies, shock, and death (35, 39). For this reason, aerosol exposure is the predicted form of delivery if BSAgs were to be used as a biological weapon against civilian

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 APR 2005	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Human leukocyte antigen-DQ8 transgenic mice: a murine model to examine the toxicity of aerosolized staphylococcal enterotoxin B, Infection and Immunity 73:2452-2460	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Roy, CJ Warfield, KL Welcher, BC Gonzales, RF Larsen, T Hanson, J David, CS Krakauer, T Bavari, S	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD	**RPP-04-437**
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

The original document contains color images.

14. ABSTRACT

Staphylococcal enterotoxins (SEs) belong to a large group of bacterial exotoxins that cause severe immunopathologies, especially when delivered as an aerosol. SEs elicit the release of lethal amounts of cytokines by binding to major histocompatibility complex (MHC) class II and cross-linking susceptible T-cell receptors. Efforts to develop effective therapeutic strategies to protect against SEs delivered as an aerosol have been hampered by the lack of small animal models that consistently emulate human responses to these toxins. Here, we report that human leukocyte antigen-DQ8 (HLA-DQ8) transgenic (Tg) mice, but not littermate controls, succumbed to lethal shock induced by SEB aerosols without potentiation. Substantial amounts of perivascular edema and inflammatory infiltrates were noted in the lungs of Tg mice, similar to the pathology observed in nonhuman primates exposed by aerosol to SEB. Furthermore, the observed pathologies and lethal shock correlated with an upsurge in proinflammatory cytokine mRNA gene expression in the lungs and spleens, as well as with marked increases in the levels of proinflammatory circulating cytokines in the Tg mice. Unlike the case for littermate controls, telemetric evaluation showed significant hypothermia in Tg mice exposed to lethal doses of SEB. Taken together, these results show that this murine model will allow for the examination of therapeutics and vaccines developed specifically against SEB aerosol exposure and possibly other bacterial superantigens in the context of human MHC class II receptors.

15. SUBJECT TERMS

Staphylococcal enterotoxin B, human leukocyte antigen, DQ8, aerosol, toxicity, laboratory animals, transgenic mice

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**SAR**	**9**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

TABLE 1. Aerosolized SEB is nonlethal in HLA-DR2β/IEα transgenic and BALB/c mice

Dose (μg/kg)	Mice	No. live/total
240	HLA-DR2β/IEα	5/5
	BALB/c	5/5
310	HLA-DR2β/IEα	5/5
	BALB/c	5/5
	BALB/c[a]	0/10

[a] Mice were given a potentiating dose of LPS and exposed 3 h later by aerosol to SEB.

or military populations (14, 15). A realistic small animal model system is urgently needed to allow examination of candidate vaccines and therapeutics against forms of toxins that would be delivered via aerosol. Such an animal model has not been described; therefore, it is imperative to first establish a system to test preventatives and treatments for BSAg exposure. Our laboratory previously showed that HLA/human CD4 (HLA-DQ8 or HLA-DR3) double-transgenic mice lacking endogenous MHC class II and murine CD4 expression are extremely sensitive to BSAgs, without the need for additional potentiation (11, 38). In fact, these transgenic mice emulated human-like responses to parenterally administered BSAgs. Thus, it was hypothesized that HLA-DQ8 transgenic mice might provide a realistic small animal model to further increase our understanding of BSAg pathogenesis, as well as provide a viable system for determining the effectiveness of countermeasures against BSAg aerosols. In this report, we describe the potential use of human MHC class II/human CD4 transgenic mice as a model to examine the aerosol toxicity of a prototype BSAg, SEB. Pulmonary histology in transgenic mice was examined, and the results were compared to those for lung lesions of rhesus monkeys from a previous study. Additionally, we examined biomarkers of toxicity, including body temperature change, expression of proinflammatory cytokine genes in lungs and spleen, and cytokine proteins elicited in the sera of human MHC class II/human CD4 transgenic mice that were aerosol exposed to SEB. Our results support the hypothesis that human MHC class II/human CD4 transgenic mice represent a realistic transgenic model to examine therapeutics and vaccines against aerosol exposure to SEB and should serve as an appropriate bridge between in vitro studies and experiments with nonhuman primate.

MATERIALS AND METHODS

Experimental design. To determine whether transgenic mice were also susceptible to aerosols of SEB, groups of HLA-DQ8 mice were exposed to 5, 30, 60, 120, 240, or 310 μg of aerosolized SEB toxin per kg. Because BALB/c mice have been widely used to examine the pathological effects of BSAgs, in our initial experiments we incorporated these mice as controls. Groups of BALB/c or HLA-DR2β/IEα mice were also examined at the two highest doses for comparative purposes (Table 1). The controls received SEB only, received LPS only, or were given 75 μg of LPS intraperitoneally 3 h after exposure to aerosolized SEB. All mice were monitored for 21 days after SEB challenge. Thereafter, to further examine mechanisms of SEB-induced lung pathologies and death, HLA-DQ8 mice were exposed by aerosol to 120 μg of SEB per kg and were killed at 4, 10, and 24 h postexposure. At the indicated times, lungs, spleens, and blood samples were collected for determination of gene expression and secretion of proinflammatory cytokines. Full pathology examination was also performed on a subgroup of these animals.

Animals. Pathogen-free 10- to 12-week-old BALB/c (*H-2*^{d}) mice were obtained from Charles River (NCI-Frederick, Frederick, Md.). HLA-DQ8/human CD4$^+$ transgenic mice were created by microinjecting DNA fragments into embryos from C57BL/6 mice, as previously described (26, 33, 34). Briefly, insertion of HLA-DQα1*0301 and HLA-DQβ1*0302 gene fragments created the HLA-DQ8$^+$ transgenic mice. The C57BL/6 embryos were inserted into (C57BL/6 × DBA/2) F_1 mice and backcrossed to B10.M mice. The HLA-DQ8 molecule was then introduced into murine class II-negative mice by mating the H-2-negative strain (H-2 Ab$^{o/o}$) with HLA-DQ8.B10.M mice. Human CD4$^+$ Ab° mice were similarly created and subsequently crossed with HLA-DQ8$^+$ B10.M mice. HLA-DR2β/IEα mice were created based on the same strategy and were used as littermate controls. All mice had no detectable (<1:50) serum titers against SEA, SEB, and SEC1 as measured by enzyme-linked immunosorbent assay (ELISA).

Mice were maintained under pathogen-free conditions and fed laboratory chow and water ad libitum. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Reagents. Lyophilized SEB toxin was obtained from Porton Down (Wiltshire, United Kingdom). The toxin was over 95% pure as determined by polyacrylamide gel electrophoresis. N-terminal protein sequencing further showed that SEB was the only enterotoxin present in the preparation, and no other SEs or toxic shock syndrome toxin 1 was detected. The toxin was reconstituted before aerosolization with freshly prepared endotoxin-free phosphate-buffered saline (PBS). The SEB stock solution concentration was determined by protein assay (MicroBCA; Pierce, Rockford, Ill.). The endotoxin levels in the SEB stock were less than the detection limit, as determined by the *Limulus* amebocyte lysate QCL-100 endotoxin assay according to the instructions of the manufacturer (Biowhittaker, Frederick, Md.). *Escherichia coli* LPS (O55:B5) (Difco Laboratories, Detroit, Mich.) was reconstituted in sterile PBS and stored at −70°C.

Aerosol exposure system and aerosol characterization. Mice were exposed to SEB aerosols by using a whole-body dynamic exposure chamber housed within class III biological safety cabinets maintained under negative pressure (27, 28). Mice were contained in the whole-body chamber by four smaller, stainless-steel mesh cages. Each cage contained up to 10 mice, for a maximum of 40 mice per exposure. The total flow through the chamber was 19.0 ± 0.5 liters/min, and the pressure inside the chamber was maintained equal to that inside the safety cabinet. Aerosols were generated with a Collison nebulizer (BGI USA, Inc., Waltham, Mass.). Particle sizing of the experimental atmosphere generated revealed a mass mean aerodynamic diameter of 1 μm with a geometric standard deviation of 1.4. The exposure concentration was determined by constantly sampling the chamber with an all-glass impinger (Ace Glass, Vineland, N.J.). PBS with antifoam A (0.001%, wt/vol) (Sigma, St. Louis, Mo.) was used as the collection medium in the impinger. Starting solutions and all-glass impinger samples were measured by protein assay (Pierce MicroBCA).

The presented inhalation dose was determined by using the respiratory minute volume (V_m). The estimates were derived by Guyton's formula, expressed as $V_m = 2.10 \times W_b^{0.75}$, where W_b is body weight in grams (for mice we used the average of group weights on the day of exposure) (27, 28). The presented dose was then calculated by multiplying the total volume (V_t) of experimental atmosphere inhaled by each animal ($V_t = V_m \times$ length of exposure) by the empirically determined exposure concentration (C_e) (presented dose = $C_e \times V_t$).

Head-only aerosol challenges of rhesus macaques, performed as part of other studies (8, 9), were done in a manner similar to that for the mice with respect to particle size distribution, sampling, and toxin preparation. Primates received a presented aerosol dose of 5 to 7 50% lethal doses (LD_{50}) of SEB.

Telemetric temperature analysis. Implantable programmable temperature transponders (IPTT-100) were purchased from BioMedic Data System Inc. (Seaford, Del.). Individually sterilized transponder chips were implanted subcutaneously at least 2 weeks before initiation of the experiment. Mice were exposed by aerosol to approximately 120 μg of SEB per kg, and temperature was monitored via telemetry and recorded every hour.

Histopathology. Scheduled necropsies of control or transgenic mice were performed on the mice at 3 days postexposure. The lungs were fixed in 10% neutral-buffered formalin, routinely processed, cut at 5 to 6 μm, and stained with Mayer's hematoxylin and eosin. The lungs from two naive age-, sex-, and strain-matched controls were similarly processed and evaluated for comparative purposes.

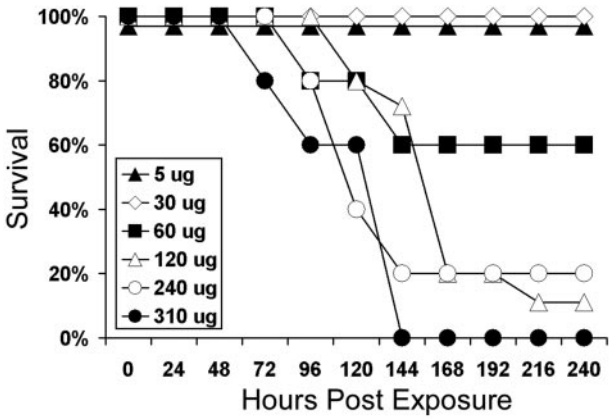

FIG. 1. Aerosol exposure to SEB is lethal to HLA-DQ8/human CD4 transgenic mice. HLA-DQ8/human CD4 transgenic mice were exposed to 5, 30, 60, 120, 240, or 310 μg of aerosolized SEB per kg. All mice were monitored for illness for 21 days after SEB challenge. Results are plotted on Kaplan-Meier survival curves as percent survival for each group over time ($n = 10$ to 20 per group). The data presented are a compilation of those from two to four experiments with each dose of SEB.

Cytokine analysis. To determine cytokine gene expression levels in tissues, total RNA was prepared from the spleen and lung tissues (Qiagen RNA Isolation Midi-Kit). The isolated RNA was reverse transcribed to produce cDNA (Invitrogen, Carlsbad, Calif.). After amplification, PCR products were separated by electrophoresis on a 1.5% agarose gel. In all reverse transcription PCR (RT-PCR) experiments, the β-actin region was amplified and served as an internal control. The protein levels of the cytokines TNF-α, IL-6, and IFN-γ in pooled samples from four to six mice per time point were determined by ELISA according to the manufacturer's specifications (Quantikine murine immunoassay kits; R&D Systems, Minneapolis, Minn.).

Statistics. Survival data were analyzed by the probit method (LD_{50} estimation) and nonparametric life-table methods by using the Mantel-Cox statistic (BMDP Dynamic, version 7.0; SAS Corp., Cary, N.C.). Descriptive statistics (mean ± standard deviation) were used to display the results of the serum cytokine analysis.

RESULTS

HLA-DQ8 transgenic mice succumb to SEB aerosol exposure. Previously, we reported that human MHC class II/human CD4 transgenic mice deficient in endogenous murine MHC class II and murine CD4 receptors were extremely sensitive to BSAgs, without the administration of potentiating agents (11, 38). In these mice, parenteral challenge with the BSAg caused in vivo proliferative responses, surges of proinflammatory cytokines in serum, expansion and retraction of specific T cells, and death (10, 11, 38). Figure 1 shows the results of the determination of the lethal dose in the transgenic mice. Previously, we showed that littermate transgenic HLA-DR2β/IEα mice respond to BSAgs in a manner similar to that of BALB/c or C57BL/6 mice (11, 38). HLA-DR2β/IEα mice, on the same genetic background and transgenic for only the β chain of human MHC class II receptor, were used as the controls for the HLA-DQ8 mice. Because BALB/c mice have been widely used to examine the pathological effects of BSAgs, in our initial experiments we incorporated these mice as controls (Table 1). Within hours after SEB exposure, all HLA-DQ8 transgenic mice were visibly distressed and showed signs of ruffled coat and lethargy. These signs of malaise persisted for 6 to 72 h after aerosol challenge or until death. As shown in Fig. 1, most of the lethality in the HLA-DQ8 mice occurred within the first 4 to 6 days after exposure to SEB. SEB administered at the highest dose (310 μg/kg) was 100% lethal to all HLA-DQ8 transgenic mice. When HLA-DQ8 transgenic mice were exposed to 240 or 120 μg of SEB per kg, 88 and 80% lethality was observed, respectively, while 60 μg/kg induced lethality in 40% of the mice. Mice were slower to succumb to SEB challenges of 120 and 60 μg/kg. The dosage of 30 or 5 μg of SEB per kg resulted in no lethality; nevertheless, the mice displayed signs of malaise and were visibly distressed for 12 to 48 h postexposure. Based on the lethality curves, the median dose resulting in 50% lethality was approximately 70 μg/kg, showing that HLA-DQ8 transgenic mice are highly susceptible to aerosols of SEB.

In sharp contrast to the case for HLA-DQ8 transgenic mice, SEB at 310 or 240 μg/kg was not lethal to HLA-DR2β/IEα mice (Table 1). The HLA-DR2β/IEα mice showed no signs of stress after aerosol exposure. These results demonstrate that even at high doses of SEB, both chains of HLA-DR are needed for toxin to be effective in the mouse model. As previously demonstrated by LeClaire et al. (21), we also observed no lethality in BALB/c mice exposed to SEB at 310 μg/kg, although 100% lethality was produced in the BALB/c mice when the aerosol exposure was combined with a potentiating dose of LPS (Table 1). As expected, lower doses of SEB or LPS given alone were not lethal to BALB/c mice (data not shown).

SEB aerosols induce lung pathology. One of the major concerns regarding SEB in aerosol form is its ability to produce severe lung damage. Enterotoxin-producing *S. aureus* strains are associated with toxic shock syndrome, which includes systemic and respiratory complications such as cardiomyopathy, renal failure, electrolyte imbalances, disseminated intravascular coagulation, encephalopathy, and adult respiratory distress syndrome (2, 13, 18, 25, 31). Since in past studies SEB aerosol exposure was shown to induce lethal pulmonary lesions in nonhuman primates, we sought to examine whether aerosol delivery of SEB to the HLA-DQ8 mice induced respiratory damage (35, 39). Therefore, the effects of SEB on the lungs of HLA-DQ8 transgenic mice were examined and compared to those histological lesions induced in rhesus monkeys, a well-established nonhuman primate model for aerosol exposure to SEs. In contrast to mice sham exposed to saline, the mice exposed to SEB aerosols displayed substantial lung damage, which was characterized by the filling of alveolar spaces, interstitium, and lymphatics with edema, fibrin, and a cellular infiltrate composed of lymphocytes, macrophages, and neutrophils (Fig. 2A and B). The pathological changes observed in the HLA-DQ8 mice that were exposed to SEB aerosols were similar to those found in rhesus monkeys exposed to lethal doses of this toxin by the same route of exposure (Fig. 2C and D).

Aerosol exposure to SEB elicits proinflammatory cytokines. Acute induction of proinflammatory cytokine genes and secretion of proteins, including TNF-α, IL-1, IL-6, and IFN-γ, in the sera and spleens of mice parenterally administered SEB has been reported, and these manifestations are closely associated with toxin-induced lethal shock (32). Although there is a wealth of information available on cytokine induction after parenteral administration of BSAgs, little is known regarding the modulation of proinflammatory cytokines in the blood,

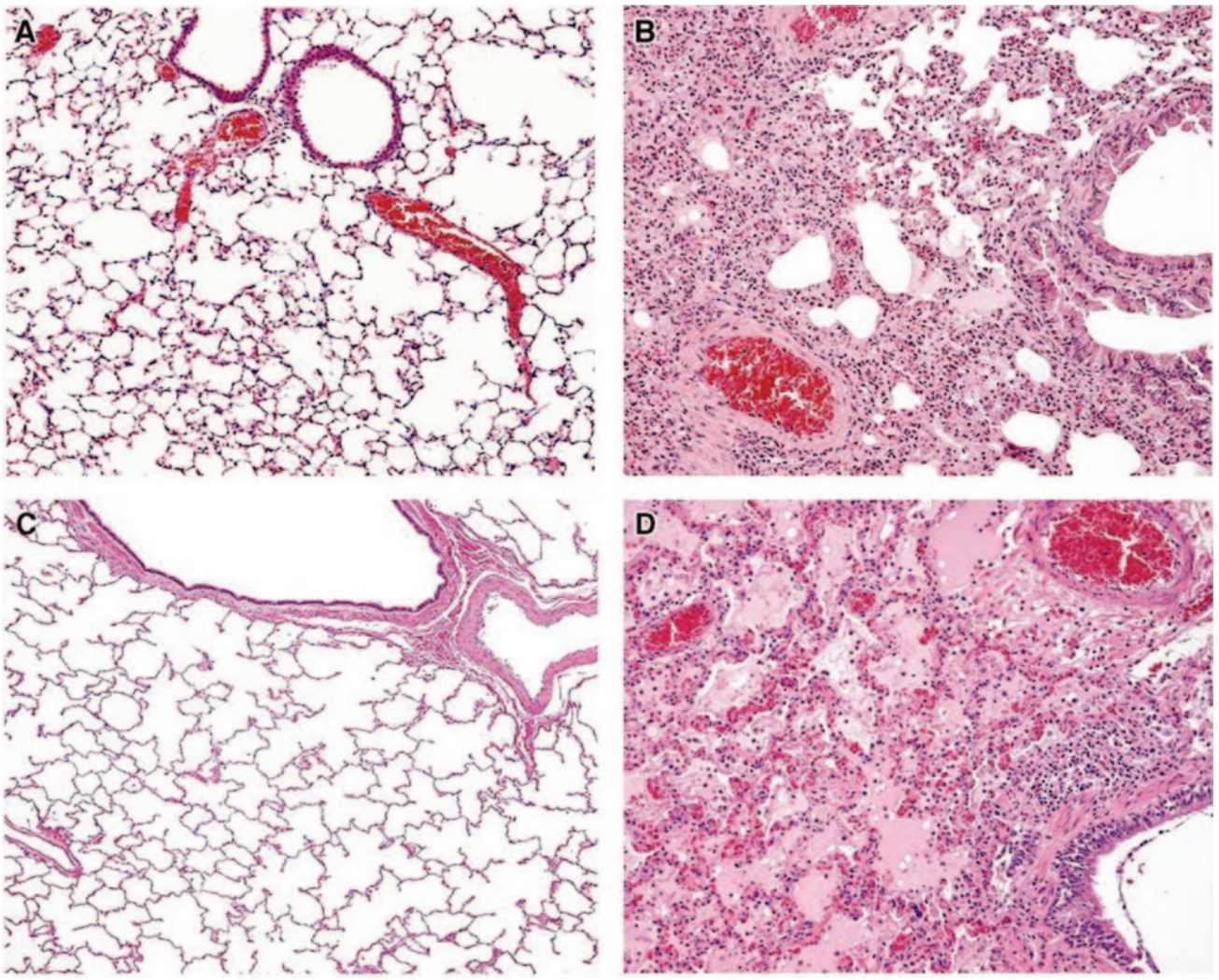

FIG. 2. Aerosol exposure to SEB induces pathological lesions in HLA-DQ8 transgenic mice. (A) Lung from a normal HLA-DQ8/human CD4 mouse. (B) Lung from an HLA-DQ8/human CD4 mouse 3 days after exposure by aerosol to 120 μm of SEB per kg. (C) Lung from a normal rhesus monkey. (D) Lung from an SEB-exposed rhesus monkey 4 days postchallenge with 5 to 7 LD_{50} of SEB delivered by aerosol. Hematoxylin and eosin staining was used. Magnification, ×200. These data are representative of findings from two animals, and the experiment was repeated twice with similar results.

lungs, and spleen after SEB aerosol challenge. Analyses of these tissue and fluid samples indicated an intense and rapid response to SEB, as several proinflammatory genes were found to be highly elevated in the lungs and spleens of HLA-DQ8 mice. The lungs of the HLA-DQ8 mice showed increases in TNF-α mRNA at all of the time points tested except in untreated mice, in which no cytokine mRNAs were detected (Fig. 3 and data not shown). The TNF-α mRNA induction stayed at high levels even at 24 h after SEB aerosol challenge, while increases in the cytokine mRNA levels of IL-2, IL-4, IL-6, and IFN-γ were not observed in the lungs of SEB-exposed HLA-DQ8 mice (Fig. 3). HLA-DR2β/IEα mice challenged with aerosol SEB showed only transient increases in IL-2, IL-4, IL-6, and IFN-γ mRNAs in the lungs (Fig. 3). The SEB-exposed HLA-DR2β/IEα mice had sustained levels of TNF-α, which were similar to those detected in HLA-DQ8 mice (Fig. 3). In contrast to the case for lungs, increased levels of TNF-α mRNA were noted at 4 and 10 h postexposure but were not detected at 24 h in spleens of SEB-exposed HLA-DQ8 mice (Fig. 4). The mRNA levels of IL-2 and IFN-γ increased in the spleens of SEB-exposed HLA-DQ8 transgenic mice after 4 h and remained elevated at 10 and 24 h after SEB exposure (Fig. 4). Increases in the mRNA expression of IL-6 genes were detected at 4 and 10 h postexposure but had subsided by 24 h after SEB aerosol challenge of the HLA-DQ8 mice (Fig. 4). We observed no enhancement of any cytokine mRNA prior to intoxication or of IL-4 mRNA throughout the experiment in the transgenic mice (Fig. 4). HLA-DR2β/IEα mice challenged with aerosol SEB showed only transient increases in IL-2 and TNF-α mRNAs in their spleens and no detectable mRNA expression of IL-4 or IL-6 (Fig. 4). IFN-γ mRNA expression was induced at 10 and 24 h after exposure to aerosolized SEB in the HLA-DRβ/IEα mice (Fig. 4).

Since elevations in the mRNA expression of the cytokines

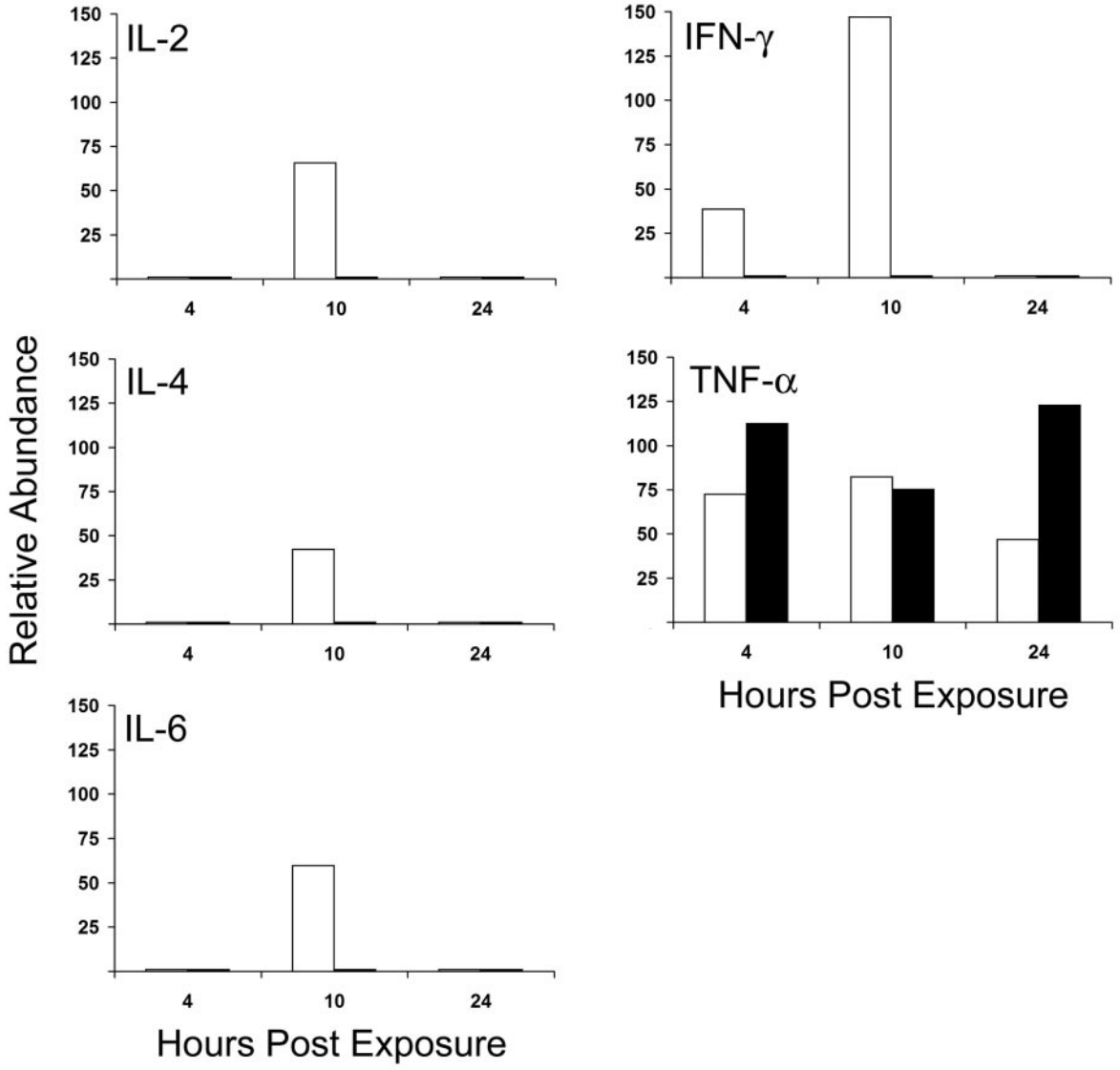

FIG. 3. Exposure of HLA-DQ8 mice to aerosolized SEB induces cytokine mRNA changes in the lungs. Total RNA was isolated from the lungs of HLA-DR2β/IEα (unfilled bars) or HLA-DQ8 (filled bars) mice exposed by aerosolization to 120 μg of SEB per kg after 4, 10, and 24 h. RNAs from three to five animals were pooled, reverse transcribed, and cDNA amplified for IL-2, IL-4, IL-6, IFN-γ, TNF-α, or β-actin. RT-PCR products were resolved on an ethidium bromide-stained agarose gel. The data are expressed as the relative abundance of each cytokine mRNA, which was determined by comparing the signal of the RT-PCR product for the cytokine mRNA with that of the loading control of β-actin. These data are representative of those from three experiments with a similar design and outcome.

varied in the spleen and lungs, we examined whether there were changes in serum cytokine levels. As shown in Fig. 5, substantial amounts of IL-2 and IL-6 were detected in sera of transgenic mice 4 h after SEB exposure, but these quickly declined. A robust and long-lasting IFN-γ response was observed after SEB challenge, contrasting with no apparent elevation in detectable serum TNF-α (Fig. 5). HLA-DRβ/IEα mice showed no sign of cytokine level increases, and no remarkable elevations in serum cytokines were detected at any time after SEB exposure (Fig. 5).

Temperature changes in SEB-exposed HLA-DQ8 mice. One of the biological manifestations of toxic shock syndrome is temperature elevation (9, 17); hence, body temperature may provide a biomarker for SEB toxicity. In contrast to that in humans and nonhuman primates, toxic shock in mice is accompanied by hypothermia (37). To examine the effects of SEB exposure on the body temperature of HLA-DQ8 mice, telemetric implants were placed into the mice 14 days before SEB exposure. After SEB challenge, body temperature was continuously monitored for 14 days or until the animal died. Two days after SEB aerosol exposure, HLA-DQ8 mice receiving a dose of 120 μg/kg displayed sudden drops in body temperature (Fig. 6). The mice seemed to temporarily recover from hypothermia within 96 to 120 h after challenge, but then their body temperatures dropped swiftly within the next 12 h or until death. In these experiments, a dose of 120 μg/kg caused 100% lethality in HLA-DQ8 mice. Transgenic control HLA-DRβ/IEα mice showed no sign of hypothermia (Fig. 6) and had

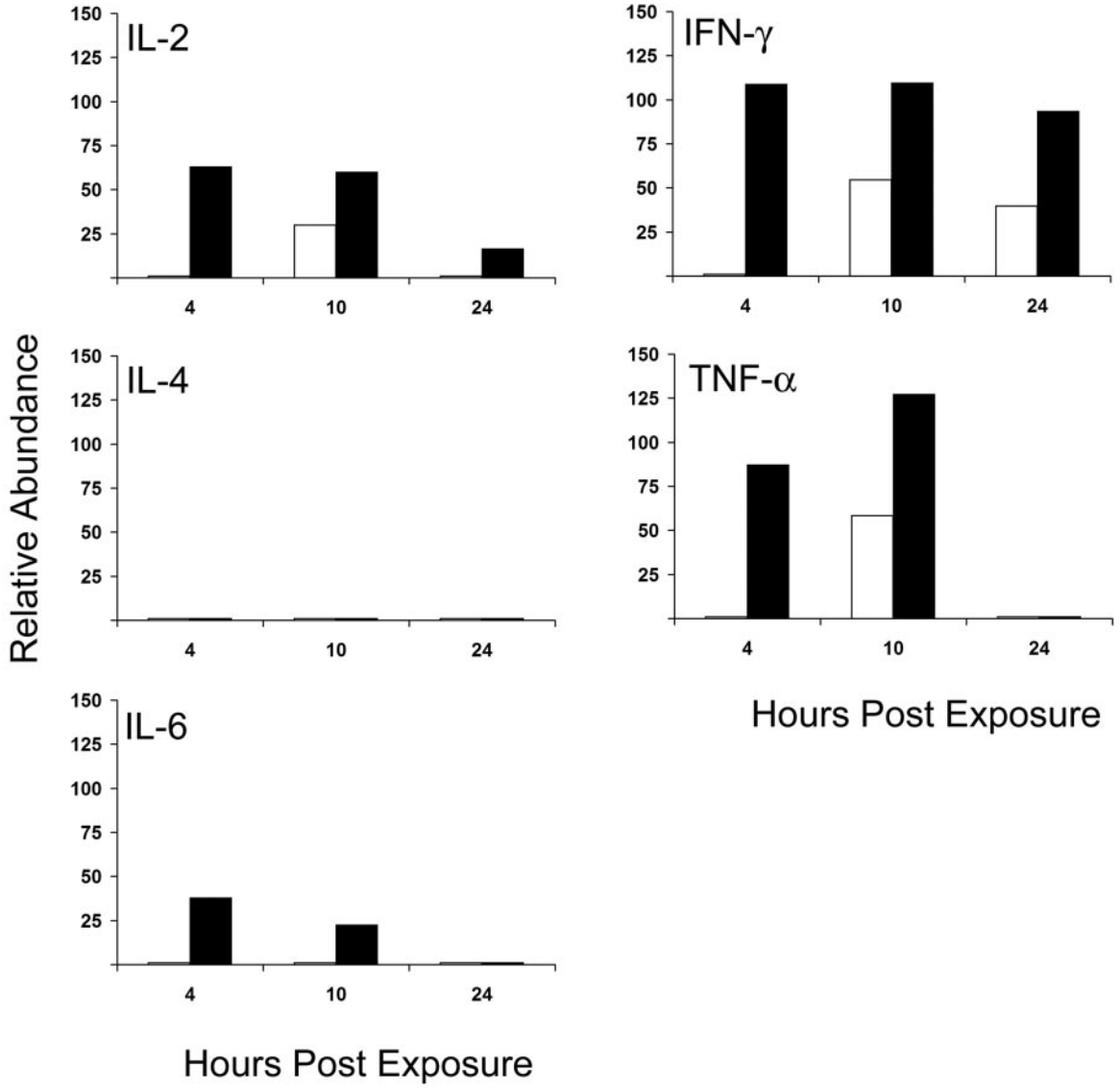

FIG. 4. Exposure of HLA-DQ8 mice to aerosolized SEB induces cytokine mRNA changes in the spleen. Total RNA was isolated from the spleens of HLA-DR2β/IEα (unfilled bars) or HLA-DQ8 (filled bars) mice exposed by aerosolization to 120 μg of SEB per kg after 4, 10, and 24 h. RNAs from three to five mice were pooled, reverse transcribed, and cDNA amplified for IL-2, IL-4, IL-6, IFN-γ, TNF-α, or β-actin. RT-PCR products were resolved on an ethidium bromide-stained agarose gel. The data are shown as the relative abundance of each cytokine mRNA, which was determined by comparing the signal of the RT-PCR product for the cytokine mRNA with that of the loading control of β-actin. These data are representative of those from three experiments with a similar design and outcome.

normal physical activity. No changes in body temperature were observed when HLA-DRβ/IEα mice were monitored for an additional 4 days (data not shown). These results suggest that, like in rhesus monkeys, temperature modulation is manifested in HLA-DQ8 mice soon after exposure to SEB aerosols, and temperature shifts may be indicative of BSAg-induced toxic shock (Fig. 6) (9).

DISCUSSION

SEs are of considerable concern as potential biological weapons (14, 15). In this study, we built on our previous data to show that mice transgenic for both αβ chains of human MHC class II receptor are highly susceptible to SEB aerosol exposure. HLA-DQ8 mice displayed pathologies similar to those observed in nonhuman primates, including severe hypothermia and a massive surge of proinflammatory responses in the lungs, spleen, and serum. Hence, our present study clearly documents the relevancy of the HLA-DQ8 transgenic mouse model for examination of the pathogenesis of SEB aerosols (and possibly other BSAgs). This HLA transgenic mouse should provide an opportunity to develop an animal model that is genetically more closely linked to humans.

Compared to humans and nonhuman primates, mice are more resistant to the immunological effects of BSAgs; however, lethal shock is observed in mice when these toxins are

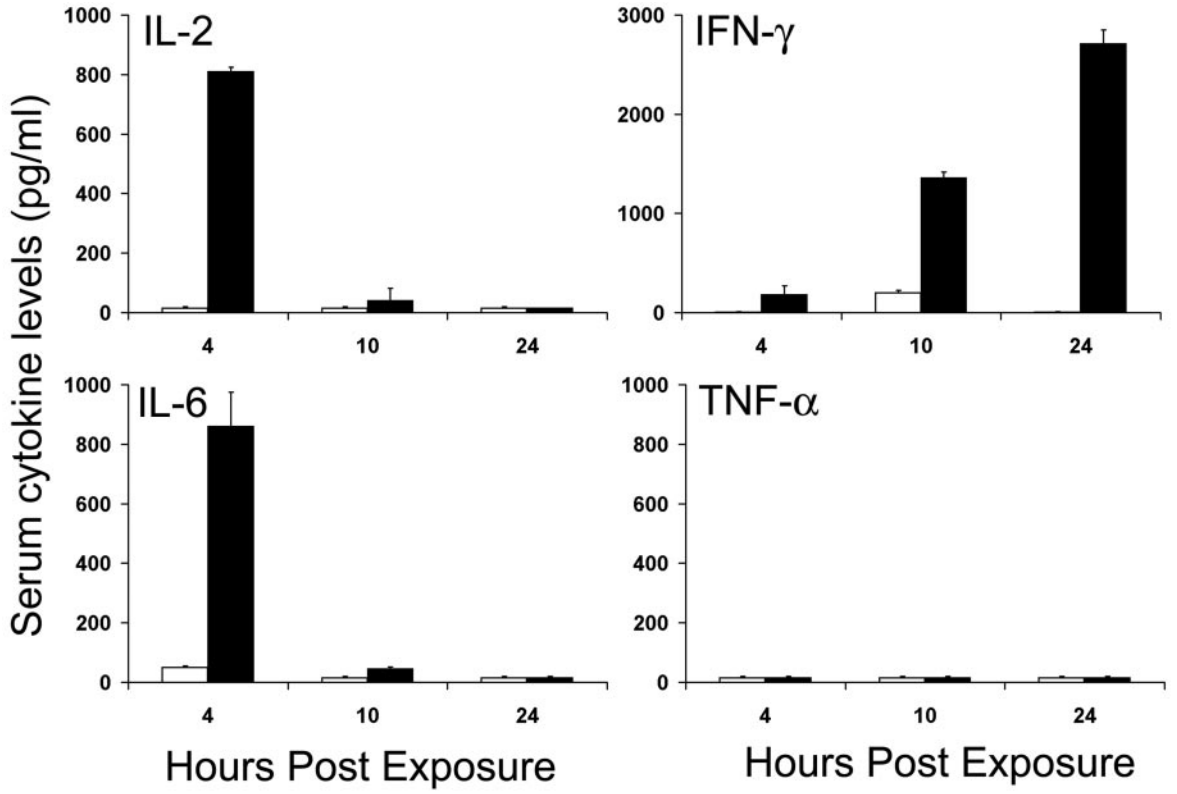

FIG. 5. Exposure to SEB aerosols increases serum cytokine levels in HLA-DQ8 mice. Circulating cytokine levels in HLA-DR2β/IEα (unfilled bars) or HLA-DQ8 (filled bars) mice were measured at 4, 10, or 24 h after exposure to aerosolized SEB (120 μg/kg). The levels of IL-2, IL-6, IFN-γ, and TNF-α were determined by ELISA. The bars indicate the means for four animals, and the error bars indicate the standard deviations. These data are representative of those from three experiments with a similar design and outcome.

administered in combination with potentiating agents such as LPS, D-galactosamine, or actinomycin D or after blockade of glucocorticoid receptors or inhibition of inducible nitric oxide synthase (23, 24, 32). Recently, Savransky et al. exposed C3H/HeJ mice to SEB intranasally and observed that SEB exposure causes toxic shock. However, no clear explainable mechanism was identified (30), primarily because of the inherent differences of murine MHC class II-elicited response to potentiation agents. It is also worth noting that other, more cumbersome models, including a chimeric mouse model and transplantation of bone marrow from SCID mice into lethally irradiated mice to enhance BSAg-induced abnormalities in mice, have been examined (1). The primary drawback to wide use of these murine models in assessing the superantigenic response is the lack of relevance to human SE pathogenesis.

One of the hallmarks of SE toxicity in humans and nonhuman primates is its ability to induce incapacitation, which may include self-limiting nausea, vomiting, and temperature elevation (2, 9). In nonhuman primate studies of experimental exposure, the most consistent sign of incapacitation is elevation in body temperature, which is most likely mediated by secretion of inflammatory cytokines; these fluctuations in body temperature have proven to be a relevant biomarker of vaccine dose effects and incapacitation (9). Previously, we used telemetric analysis to determine the superantigenicity of an attenuated SEB vaccine in rhesus monkeys, and in the same study, measurement of temperature showed substantial promise as a biomarker for predicting vaccine efficacy (8, 9). Vlach et al. have used telemetric monitoring of temperature in mice that were parenterally administered SEB and then potentiated with LPS, and they observed a correlation between death and hypothermia (37). Considering the fact that LPS also elicits the release of proinflammatory cytokines, it is not surprising that in the aforementioned study, there was a strong correlation between administration of LPS and drop in temperature. Our present studies indicate that aerosol exposure to SEB without potentiation causes incapacitation in HLA-DQ8 transgenic mice, and these results demonstrate that aerosol exposure to SEB induced a hypothermic response in HLA-DQ8 transgenic mice at 48 h after challenge. Considering the results from the present study, the contrast between elevated temperature in previous primate studies and a profound depression of body temperature in the transgenic animals indicates that correlative temperature fluctuations between species cannot be explained through the use of potentiating agents. In this context, identification of body temperature as a correlate biomarker of toxic shock may be an important tool and may be used to distinguish differences in vaccine dosing regimens or various therapeutics and to bridge nonhuman data to eventual human preparations (9). Further studies will be needed to identify the specific mediators involved in temperature modulation.

A synergistic action of several proinflammatory cytokines, such as TNF-α, IFN-γ, and IL-6, is probably responsible for BSAg-induced lethal shock. Several studies using the potenti-

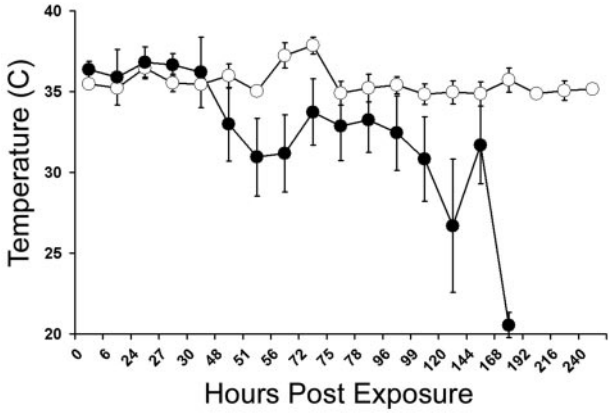

FIG. 6. Exposure of HLA-DQ8 mice to aerosolized SEB results in dramatic decreases in body temperature. Mice were implanted with telemetric chips to measure their body temperature. HLA-DRβ/IEα (open circles) or HLA-DQ8 (filled circles) transgenic mice were exposed to 120 μg of SEB per kg. The body temperatures of the mice were monitored after exposure. The results are plotted as the mean of the body temperature ($n = 5$ per group, except at time points beyond 120 h, when the SEB-treated HLA-DQ8 mice began to succumb to toxic shock). The error bars indicate the standard deviations of the group means. These data are representative of those from three experiments with a similar design and outcome.

ated mouse models for SEB have shown that TNF may be a critical player in the initiation of generalized lethal toxic shock. In contrast, using a more relevant animal model, it was reported that rhesus monkeys exposed to SEB aerosols had abundant amounts of IFN-γ, IL-6, and IL-2 without appreciable amounts of serum-born TNF (19). The levels of these cytokines in serum were observed within 4 h after SEB exposure. Importantly, the HLA-DQ8 transgenic mice had a burst of IFN-γ, IL-2, and IL-6 serum responses but no detectable TNF-α responses in serum after aerosol exposure to SEB. In contrast to parenteral administration of SEB (18, 24, 32), aerosol challenge of HLA-DQ8 transgenic mice with SEB induced no detectable circulating TNF-α. Hence, our present working hypothesis is that lung toxicity of SEB, when delivered by the aerosol route, is linked to local lung TNF-α responses, while the systemic lethal shock is driven by elicited IFN-γ.

Here, we showed that SEB also induced a cytokine proinflammatory profile that resembled a Th1-type response and was dominated by long-lasting IFN-γ responses in serum. A number of other studies have shown that BSAgs favor the development of Th1-type cytokine responses (3, 4). For instance, parenteral exposure of HLA-DQ8 or HLA-DR3 transgenic mice to streptococcal pyrogenic exotoxin or SEB elicited high serum-borne Th1-like responses (11, 38). Furthermore, it has been shown that it may be possible to develop synthetic peptides to specifically blunt SE-induced Th1 cytokine responses (4, 36). In one such study, it was shown that direct peptide binding to MHC possibly blocks the superantigen binding site; however, competition experiments were not reported (36). The possibility that a conserved region in the superantigens contains an epitope capable of providing MHC protection against a significant subset of these toxins may provide a generally useful vaccine or possible therapeutics. Whether such peptides can be adequately protective against aerosol exposure to SEs needs to be evaluated in the context of HLA-DQ8 transgenic mice.

A multitude of cells and mediators participate in the acute inflammatory response that characterizes SAg toxicity and SAg-induced lethal shock. As a result, choosing a BSAg therapy that targets a single cytokine such as TNF or IFN-γ might seem less than desirable. Instead, inhibition of MHC class II and T-cell receptor binding to SAgs by antibody might be a more realistic therapeutic approach. Another, more direct method would be to identify small-molecule (nonpeptide) compounds that inhibit SE interaction with human MHC class II or T-cell receptor. Initially, compounds could be tested by using an in vitro method, and the identification and development of SE therapeutics could be guided by structure-based molecular modeling. After in vitro studies, the mouse model described in this report would be an effective and cost-efficient means for testing the in vivo efficacies of lead compounds, as it uses HLA as the target receptor. In support of this particular route for inhibiting SE activity, molecular modeling has been used to identify several inhibitors of CD4-MHC class II binding inhibitors which have been shown to be efficacious in autoimmune disease and allograft transplant rejection (12, 22, 29).

Results from previous studies clearly demonstrated that anti-SEB antibodies are the main protective factors against SEB intoxication (8, 11, 20). Most adult primates have been exposed to *S. aureus* or have encountered low environmental doses of SEs and consequently have anti-SE antibodies (6, 20). This may be of concern when examining the efficacy of therapeutic antibodies or vaccines against SEs in these animals. However, *S. aureus* is not a common mouse pathogen, and not surprisingly, screening of more than 1,000 mice sera indicated that none had anti-SE antibodies (data not shown). Thus, the model described in these studies can be used to screen the efficacy of anti-SE antibody against aerosol exposure without concerns about background antibody responses.

In summary, the mechanism of aerosol exposure to SEB and subsequent toxicity has not been completely resolved; however, there are striking similarities in the pathological lesions, cytokine responses, and acute dose response between the potentiated mouse, the nonhuman primate, and the HLA-DQ8 transgenic mouse models (21, 38). Primate MHC class II molecules (especially human class II molecules) are the evolutionary target for bacterial superantigens and thus have a much higher affinity for superantigens than do mouse MHC class II molecules. This is further indicated by the need to potentiate the inbred mouse in order for the pathological changes to mimic those of the nonhuman model or those seen in humans. Furthermore, it is especially critical to determine immune correlates of protection in animal models that emulate human responses to predict the efficacy of human vaccines and therapeutics, as conventional field trials of protection against aerosol exposure to SEs are not possible. In this context, the HLA-DQ8 transgenic mouse model can be used as a system to identify and validate biomarkers and test lead vaccines and therapeutics against aerosol exposure to BSAgs. The pathological changes observed in SEB-intoxicated mice suggest that this transgenic rodent may be a suitable bridge between in vitro models and nonhuman primate testing, providing a lower species model to further study SE pathogenesis and test candidate

therapeutic compounds and vaccines. To this end, we are currently performing temporal pathogenesis studies to fully characterize the pathology of superantigen-induced lesions in HLA-DQ8 mice.

ACKNOWLEDGMENTS

This work was supported by funds from the U.S. Army Medical Research and Materiel Command (project 02-4-3T-053). K.L.W. is the recipient of a National Research Council Fellowship. NIH grant AI14764 supported the production of the transgenic mice.

We thank Paul Zhou for producing the DR2 mice, Shen Cheng for the DQ8 mice, Chris Benoit and Diane Mathis for the Ab^o mice, Tak Mak for the CD4^o mice, and Richard Flavell for the human CD4 transgenic mice.

The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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Editor: J. D. Clements